

REMARKS

In this REMARKS section, the following topics are addressed.

1. Examiner Fernandez objecting to the specification with respect to the recitation in page 19, lines 3-15 of "between the plates having 10 million cells or more".
2. Examiner Fernandez objecting to the specification with respect to the recitation in page 27, lines 24-31 of "having 10 million cells or more".
3. Examiner Fernandez objecting to the specification with respect to the recitation in page 19, lines 3-15 of "per 10 milliliters".
4. General Discussion of Applicant's Claimed Invention and Prior Art.
5. Examiner Fernandez rejecting the Applicant's claimed invention under 35 USC § 103 as being unpatentable over Meserol (5,720,921) and/or Examiner Fernandez rejecting the Applicant's

claimed invention under 35 USC § 103 as being unpatentable over Meserol (5,720,921) in view of Hibi et al (4,800,163).

6. Additional Remarks

1. Examiner Fernandez objecting to the specification with respect to the recitation in page 19, lines 3-15 of "between the plates having 10 million cells or more".

The Applicant's representative would like to point out that the language that Examiner Fernandez objects to as being "new matter" is in fact in the original PCT International Patent Application as follows:

"The vesicles can be living cells, and the medium can be a physiological medium and has a conductivity between 50 and 500 μ S/cm. The number of living cells that are treated in the chamber at one time can be more than 10 million in number. Furthermore, the number of living cells that are treated in the chamber at one time can be more than 20 million in number." (at page 20, lines 14-20.

In view of the above, it is respectfully requested that Examiner Fernandez withdraw her objection.

2. Examiner Fernandez objecting to the specification with respect to the recitation in page 27, lines 24-31 of "having 10 million cells or more".

The Applicant's representative would like to point out that the language that Examiner Fernandez objects to as being "new

matter" is in fact in the original PCT International Patent Application as follows:

"The vesicles can be living cells, and the medium can be a physiological medium and has a conductivity between 50 and 500 μ S/cm. The number of living cells that are treated in the chamber at one time can be more than 10 million in number. Furthermore, the number of living cells that are treated in the chamber at one time can be more than 20 million in number." (at page 20, lines 14-20.

In view of the above, it is respectfully requested that Examiner Fernandez withdraw her objection.

3. Examiner Fernandez objecting to the specification with respect to the recitation in page 19, lines 3-15 of "per 10 milliliters".

The Applicant's representative would like to point out that the language that Examiner Fernandez objects to has already been deleted in the previously filed document entitled SECOND ELECTION OF SPECIES AND AMENDMENT which was filed on 09/22/2008.

4. General Discussion of Applicant's Claimed Invention and Prior Art.

In the Applicants specification, there is both a broad and a detailed discussion of Prior Art in the field of electroporation, as the Prior Art relates to problems created, but not solved, in the Prior Art. In this respect, the Applicants specification lists and discusses a large number of Prior Art U. S. patents in the specification of the PCT International Patent Application at

pages 10-16, with special attention directed to TABLE 4 on page 11.

As a matter of interest, Meserol (5,720,921), which is cited by Examiner Fernandez and discussed further hereinbelow, is cited in the Applicants specification in TABLE 4 on page 11 and is discussed on page 14, lines 20-29.

More specifically, the Applicants specification lists and discusses Prior Art in both flow electroporation and static electroporation. See TABLE 4 on page 11. It is important to note that the Prior Art discussed does not disclose sequential batch electroporation as provided by the Applicants claimed invention (see Claim 19).

The Applicants specification discloses FORMULAS 1, 2, and 3 which are taken from Electroporation and Electrofusion in Cell Biology, edited by Eberhard Neumann, Arthur Sowers, and Carol Jordan, Plenum Press, 1989. See page 4 of the PCT International Patent Application.

At this point it is important to point out that in Claim 1 of the Applicant's claimed invention, the "geometric factor" in (cm^{-1}) is defined by the Applicants as the quotient of the electrode gap squared (cm^2) divided by the chamber volume (cm^3). It is also important to point out that this Applicant-defined "geometric factor" is inventively evolved from the Prior Art

FORMULA 3. The Prior Art itself does not define the "geometric factor" which is a creation of the present Applicants.

In the Applicants specification, there are discussions of routine experimentation which have been attempted to increase electroporation scalability without creating undesirable results, such as an undesirable amount of heat. However, as pointed out in the Applicants specification, such routine experimentation has not led to successful results. Only the Applicant's claimed invention leads to the Applicants successful results.

As a general summary, there is no claim that the Applicant's invention covers all electroporation. There is no claim that the Applicant's invention covers all static electroporation. There is no claim that the Applicant's invention covers all scaling up of electroporation methods. There is no claim that the Applicant's invention precludes routine experimentation in scaling up electroporation that is in accordance with a person having ordinary skill in the art.

However, the Applicant's claimed invention covers methods of scaling up static electroporation that are beyond the Prior Art and beyond routine experimentation in scaling up a static electroporation method.

Stated somewhat differently, there are two fundamental concepts (teachings) of this invention:

The first fundamental concept of the invention is to use a specific combination of Applicant-defined "geometric factor" and suspension conductivity and electrical resistance to avoid excessive heating of the suspension which damages or kills the cells to be transfected. This relationship of the invention is constrained by the triangular graph in FIG 2.

The Applicant-defined "geometric factor" cannot be discussed without simultaneously discussing suspension conductivity. The graph in FIG. 2 specifies which of the Applicant-defined "geometric factors" can be used with a specific suspension conductivity. The two metrics cannot be set independently. This present invention is designed for large scale commercial applications with low maintenance requirements.

The second fundamental concept of the invention is static processing. The chamber as described by the Applicant-defined "geometric factor" and suspension conductivity has the following desirable attributes:

1. Reduces heating by imposing a suspension treatment time duty cycle. The chamber is loaded with the suspension (no electric field), the suspension is treated and the suspension is emptied from the chamber (no electric field).
2. The suspension is treated uniformly by the electric fields because it is not moving as in flow systems.

3. The electric fields used to treat the cell suspension can be complex (many different types of pulse parameters) since the cell suspension is not moving. The electric field on-times are not constrained by the flow rates in flow systems.

Now, here are Remarks which directly relate to amendments to the claims made in this AMENDMENT.

Currently amended claim 1 is as follows:

1. (Currently amended) A method of treating vesicles with exogenous material for insertion of the exogenous material into the vesicles, comprising the steps of:

a. statically retaining the vesicles and the exogenous material in a medium in a suspension in a treatment volume in a chamber which includes electrodes, wherein the chamber has a geometric factor (cm^{-1}) defined by the quotient of the electrode gap squared (cm^2) divided by the chamber volume (cm^3),

wherein said geometric factor is less than or equal to 0.1 cm^{-1} ,

wherein the suspension of the vesicles, the exogenous material, and the medium is adjusted, such that the suspension in the treatment volume in the chamber has

conductivity in a range spanning 0.001 to 100
milliSiemens/cm,

wherein the resistance of the suspension in the chamber
is greater than one ohm,

wherein the suspension is enclosed in the chamber
during treatment, and

b. treating the suspension enclosed in the chamber
with one or more pulsed electric fields,
wherein in accordance with a. and b. above, the
treatment volume of the suspension is scalable.

With respect to "statically retaining", here are portions of
the original PCT International Patent Application that support
this amended claim language:

"To achieve the foregoing and other advantages, the present
invention, briefly described, provides a static chamber with
large volume to insure all cell are subject to the same electric
field intensity and direction and the density of the cells and
material are uniform." (on page 19, lines 3-7, of PCT
International Patent Application)..

"The method of the invention can be carried out in
sequential batches." (page 21, lines 6-7, in PCT International
Patent Application).

"The present invention uses an electrode with large capacity in combination with an electroporation buffer of defined low conductivity. This process exposes all cells to the same treatment conditions, provides control over the amperage required and can process large numbers of cells. Since the cell suspension statically remains in the chamber during application of pulsed electric fields, complex waveforms can be used."
(page 25, lines 25-32, in PCT International Patent Application).

"Another aspect of the invention further increases capacity by alternately filling and emptying the gap between the electrodes. In this manner, all desired properties are met during specific treatment and the electrodes can be re-used for subsequent treatments in an intermittent batch process." (page 25, lines 33-38 in PCT International Patent Application).

"The invention uses a static chamber with large volume to insure that all cells are subject to the same electric field intensity and direction and the density of the cells and treating material are uniform." (page 27, lines 24-27, in PCT International Patent Application).

With respect to "in the treatment volume in the chamber", this language is added to Claim 1 to remind the claim reader of the location of the suspension set forth in paragraph a..

5. Examiner Fernandez rejecting the Applicant's claimed invention under 35 USC § 103 as being unpatentable over Meserol (5,720,921) and/or Examiner Fernandez rejecting the Applicant's claimed invention under 35 USC § 103 as being unpatentable over Meserol (5,720,921) in view of Hibi et al (4,800,163).

First, the Applicant's representative points out the following chronology:

- a. Hibi et al (4,800,163) has a priority date in 1986.
- b. Meserol (5,720,921) has a priority date in 1995.
- c. The Applicants invention has a priority date in 2003.

More specifically, Hibi et al (4,800,163) precedes Meserol (5,720,921) by approximately 9 years and precedes the Applicants invention by approximately 17 years.

Also, Meserol (5,720,921) follows Hibi et al (4,800,163) by approximately 9 years and precedes the Applicants invention by approximately 8 years.

In these respects, the Applicants invention follows Hibi et al (4,800,163) by approximately 17 years and follows Meserol (5,720,921) by approximately 8 years.

Now turning specifically to Meserol (5,720,921), Meserol (5,720,921) is directed to flow electroporation. Meserol (5,720,921) teaches away from "static" electroporation. More specifically, in Meserol (5,720,921), from column 5, line 28 to

column 6, line 36, Meserol (5,720,921) discusses static electroporation techniques. In discussing the unsuitability of static electroporation techniques, Meserol (5,720,921) states "over heating of the chamber" as a big problem (see column 6, line 28). These negative teachings about static electroporation appear approximately 9 years after the disclosures of Hibi et al (4,800,163) and approximately 8 years before the Applicants invention. An important point is that in the intervening 17 years between Hibi et al (4,800,163) and the Applicants claimed invention, this problem was not solved until the appearance of the Applicants claimed invention.

Since Meserol (5,720,921) dismisses static electroporation as unsuitable, and since Meserol (5,720,921) is directed to flow electroporation, Meserol (5,720,921) does not even discuss entire areas of limitations in the Applicants claimed invention. That is, Meserol (5,720,921) does not even discuss any "geometric" factor, does not even discuss conductivity of chamber contents, and does not even discuss the electrical resistance of chamber contents.

In the Office Action dated 01/06/2009, Examiner Fernandez points to column 15, lines 5-7 of Meserol (5,720,921) where she cites a disclosure of the processing of single discrete batches. However, Examiner Fernandez has not cited a key aspect of this disclosure in Meserol (5,720,921). More specifically, the

following is stated in Meserol (5,720,921): A conventional electroporation chamber may be used when the operation of the apparatus is static, namely when single discrete batches of cells are processed [emphasis added]. Since Meserol (5,720,921) predates the Applicants invention by approximately 8 years, it is clear that the "conventional electroporation chamber" disclosed in Meserol (5,720,921) cannot possibly cover the Applicants claimed invention.

Examiner Fernandez cites Meserol (5,720,921) at column 15, lines 11-12 to point out that the distance between electrodes will vary depending on the flow volume and field strength. Clearly, this aspect of Meserol (5,720,921) relates to a Meserol's continuous flow cell, not a conventional static batch cell which is clearly not preferred by Meserol.

As a matter of interest, if the Applicant-defined "geometric factor" is employed with respect to the disclosures of Meserol (5,720,921), a calculated Applicant-defined "geometric factor" turns out to be 0.27 cm⁻¹. See Exhibit A wherein the calculated Meserol data lies out of the triangular area which is employed with the Applicants invention. It is noted that original FIG. 2 closely approximates Exhibit A.

More specifically, at Col 15, line 11, of Meserol (5,720,921), the chamber gap = 7 mm or 0.7 cm. At Col 15, line 24, the chamber volume = 1.8 mL. Then, by using this data, the

Applicant-defined "geometric factor" = $(0.7)^2/1.8 = 0.27 \text{ cm}^{-1}$.
This calculation is merely of interest because Meserol (5,720,921) does not disclose any geometric factor calculation, let alone the Applicant-defined "geometric factor" as provided in the Applicants claimed invention.

As stated above, preventing overheating with scaling up is an important benefit provided by the Applicants claimed invention. The key word here is "preventing" overheating. By following the Applicants claimed invention, overheating is prevented. Therefore, no special cooling apparatus is required.

In sharp contrast, the Meserol (5,720,921) system creates quite a bit of unwanted heat. In this respect, Meserol teaches extensive cooling is required to prevent damage to the cells being transfected.

Also, Meserol (5,720,921) teaches very short on time electric fields of about 7 ms maximum. They must be short and synchronized with the flow rate (col. 13, lines 24-26).

In sharp contrast, the Applicants claimed invention provides for electric field lengthy durations, up to a second or more. Also, with the Applicants invention, no flow rate and no special synchronization apparatus are required.

Moreover, as stated hereinabove, Meserol (5,720,921) DOES NOT EVEN DISCUSS any of the following aspects of the Applicants

claimed invention: "geometric" factor; conductivity of chamber contents; and the electrical resistance of chamber contents.

It is recalled here that these key aspects of the Applicants claimed invention are set forth graphically in the Applicants Fig. 2. Meserol (5,720,921) does not provide any teaching that even remotely approximates the Applicants disclosures in Fig. 2. The silence of Meserol (5,720,921) in these key aspects of the Applicants claimed invention should not permit Meserol (5,720,921) to block the patenting of the Applicants claimed invention.

Now turning to Hibi et al (4,800,163), Examiner Fernandez states that Hibi et al (4,800,163) discloses parameters of chamber volume and electrode gap, which when calculated in accordance with Applicant-defined "geometric factor", calculate to be less than $0.1 \text{ (cm}^3\text{)}$. As far as Examiner Fernandez's statement goes, this is true.

However, Hibi et al (4,800,163) do not even discuss any of: the Applicant-defined "geometric factor"; conductivity of chamber contents; and the electrical resistance of chamber contents. That is, Hibi et al (4,800,163) do not even discuss these key aspects of the Applicants claimed invention. In view of these facts, it may be said that by using the Applicant-defined "geometric factor" and by using chamber and electrode gap

parameters in Hibi et al (4,800,163), BY MERE COINCIDENCE, the calculation results in a number that is less than $0.1 \text{ (cm}^{-1}\text{)}$, which is specified in the Applicants claimed invention. However, even though this one fact is true, Hibi et al (4,800,163) is still silent as to the nature of the Applicant-defined "geometric factor", is still silent as to the Applicant-defined calculation for the "geometric factor", is still silent as to conductivity in the chamber, and is still silent as to electrical resistance of the material in the chamber.

It is recalled here that these key aspects of the Applicants claimed invention are set forth graphically in the Applicants Fig. 2. Hibi et al (4,800,163) does not provide any teaching that even remotely approximates the Applicants disclosures in Fig. 2. The silence of Hibi et al (4,800,163) in these key aspects of the Applicants claimed invention should not permit Hibi et al (4,800,163) (either alone or in combination with Meserol (5,720,921)) to block the patenting of the Applicants claimed invention.

Furthermore, neither Hibi et al (4,800,163) nor Meserol (5,720,921) teach a formula or graphical method of scaling up. Of course, the Hibi et al (4,800,163) chamber could be scaled up in volume to be used in the Meserol (5,720,921) method. However, what is missing in Hibi et al (4,800,163) and Meserol (5,720,921) are means to scale-up the volume without the undesirable effect

of changing chamber resistance. These aspects of Hibi et al (4,800,163) and Meserol (5,720,921) are in sharp contrast with the Applicants claimed invention.

Also, Hibi et al (4,800,163) teach continuous flow electroporation and continuous flow with static cell fusion (Col 1, lines 50-55). On the other hand, the electroporation/transfection function in Hibi et al (4,800,163) is continuous flow only (Col 6 lines 31-32, 37-40). This is in sharp contrast with the Applicants invention of static batch electroporation.

In addition, Hibi et al (4,800,163) teach large volume chambers are not economical. The Hibi et al (4,800,163) chamber volumes are measured in microliters. The apparatus is not suitable for large scale commercial production (Col 6, lines 41-46). Hibi et al (4,800,163) also teach that electrotransfection should take place at 0 deg. C ((column 3, lines 49-51). This is in sharp contrast with the Applicants claimed invention which can successfully be conducted at room temperature.

More specifically, Hibi et al (4,800,163) do not teach any geometric factor, let alone the Applicant-defined "geometric factor". Also, Hibi et al (4,800,163) does not address the very important parameter of conductivity.

With respect to both Meserol (5,720,921) and Hibi et al (4,800,163), it is important to emphasize that the Applicant-defined "geometric factor" is NOT PART OF THE PRIOR ART.

More specifically, on May 4, 2009, the term "geometric factor" was searched on the Google search engine with the following search statement: "geometric factor" electroporation chamber. There were 96 hits, and there were 38 hits actually displayed. All of the displayed 38 hits were looked at, and only references to the Applicants claimed invention, by way of the Applicants U. S. Patent Application Publication No. US 2006/0108229 A1, was there a discussion of a "geometric factor" that relates to the Applicant-defined "geometric factor". Once again, clearly the Applicant-defined "geometric factor" is not part of the Prior Art.

As disclosed in the Applicants specification, on page 4 thereof, FORMULA 3 is taken from Electroporation and Electrofusion in Cell Biology, edited by Eberhard Neumann, Arthur Sowers, and Carol Jordan, Plenum Press, 1989 (hereinafter referred to as Neumann et al), which is part of the Prior Art. However, it is clear that the Applicant-defined "geometric factor", though having some similarities to FORMULA 3 from Neumann et al, has significant differences from FORMULA 3.

With an effort to see if references to Neumann et al also disclose the Applicant-defined "geometric factor", another search

was conducted on May 4, 2009 by the Applicant's representative on the Google search engine with the following search statement: Neumann Sowers Jordan "geometric factor". There were 12 hits, and 11 of these 12 hits were displayed. All displayed hits were looked at. The only hits which cited the Applicant-defined "geometric factor" were references to publications disclosing the present Applicants invention. This fact serves as additional evidence that the Applicant-defined "geometric factor" is NOT IN THE PRIOR ART.

In view of the above, it is respectfully requested that the Examiner reconsider the rejections of the Applicants claimed invention in view of Meserol (5,720,921) and/or Hibi et al (4,800,163) and allow the Applicants claims.

6. Additional Remarks

The Applicants specification has been amended in the paragraph on page 21, spanning lines 5-7, as follows:

With the ~~The~~ method of the invention, the chamber volume
is scalable ~~in a range spanning 2 to~~
~~10 above 2~~ milliliters. The method of the invention can be
carried out in sequential batches.

In addition, the Applicants the specification has been amended in the paragraph on page 27, spanning lines 14-22, as follows:

The preferred operating region of the present invention is then:

| | |
|---|--|
| Cell diameter | > 1 micrometer |
| Chamber volume | > 1 milliliter <u>2 milliliters</u> |
| Conductivity of Material to be treated | > 1 microSiemens/cm |
| Total resistance of material to be treated in chamber | > 1 ohm |
| Geometric Factor of Chamber | < 0.1 cm-1 |

Support for these amendments to the specification both in the paragraph on page 21, spanning lines 5-7 and in the paragraph on page 27, spanning lines 14-22 is found in the original PCT International Patent Application as follows:

At page 20, lines 1-2 which states:

"Preferably, the chamber has at least a 2 milliliter capacity."

At page 21, lines 31-34 which states:

"In accordance with another aspect of the invention, an electroporation apparatus is provided which includes a chamber which has a chamber volume of at least 2 milliliters."

At page 32, claim 3 which states:

"3. The method of claim 1 wherein the chamber has at least a 2 milliliter capacity."

The Applicants specification has been amended in the paragraph on page 23, spanning lines 9-13, as follows:

FIG. 2 is a graph illustrating the operating range of the method of the invention, inside the triangle, and how the operating range of the invention is outside operating ranges of prior art electroporation methods, indicated by small blocks outside the triangle. Clearly, FIG. 2 shows one side of the triangle to be a herein-defined Geometric Factor, one side of the triangle to be conductivity, and one side of the triangle to be resistance in ohms. More specifically, the conductivity is in a range spanning 0.001 to 100 milliSiemens/cm; the herein-defined Geometric Factor ranges from greater than 0.000001 cm⁻¹ to less than or equal to 0.100000 cm⁻¹; and the resistance is greater than one ohm.

This amended language serves to further describe original
FIG. 2. Therefore, no "new matter" is added by this description.

Here are some additional points.

With Continuous Flow, a cell suspension moves continuously
at a constant flow rate through the electrode producing the
electric field.

With Static Batch, the cell suspension is placed in a
chamber by some means, the electric field is applied and the cell
suspension is removed from the chamber by some means. The cell
suspension is motionless and treated uniformly by the electric
field.

With Repetitive Static Batch, the Static Batch process is
automated permitting a number of Static Batch cycles without
intervention. The cell suspension is motionless and treated
uniformly by the electric field. By selecting the optimum
Applicant-defined "geometric factor" and conductivity, in
accordance with the subject invention, a very high
production low maintenance system can be produced.

More specifically, the Static batch concepts are embodied in
the following Cyto Pulse commercial large volume transfection
systems:

Cyto LVT-S which processes up to 20 mL in a single
batch; and

Cyto LVT-P which processes 5 mL every 7 seconds in Repetitive Static Batches for an hour or more. Production volume is in liters.

These Cyto Pulse systems are used to produce polynucleotide therapeutics for human use and proteins for research use.

Turning to yet another distinction between the Prior Art and the Applicants invention, in the Applicants specification, there is extensive discussion of the problem of unwanted heat and its effects on cellular materials in electroporation. In fact, one of motivations for inventing the Applicants invention was to solve the problem of unwanted heat generation during electroporation of cellular material.

Successfully, the method of the Applicants claimed invention prevents the generation of unwanted heat. Even though the method of the Applicants invention is carried out at room temperature, the occurrence of unwanted heat is prevented by the Applicants invention without the need for an extraneous cooling system.

This prevention of the occurrence of unwanted heat with the Applicants invention, without the need for an extraneous cooling system, is in sharp contrast with the handling of the heat problem with either Meserol (5,720,921) or Hibi et al (4,800,163).

More specifically, with Meserol (5,720,921), unwanted heat is generated during electroporation, and an extraneous cooling system (including cooling coil 68) is provided to take away the unwanted heat.

More specifically, with Hibi et al (4,800,163), the electrotransfection is carried out at 0 deg. C. (column 3, lines 49-51). In this respect, Hibi et al (4,800,163) uses some sort of cooling system in order to reduce the temperature of electroporation from room temperature to 0 deg. C..

Therefore, the handling of unwanted heat is another clear distinction between the Applicants claimed invention and the Prior Art of Meserol (5,720,921) and Hibi et al (4,800,163).

Please note that the following claims are currently in the case: 1 to 6, 8, 16, 19 to 22, 24, 25, 28, 29, 31, 35, 38, 39, 42, and 45.

The following claims have been cancelled: Claims 7, 9 to 15, 17, 18, 23, 26, 27, 30, 32 to 34, 36, 37, 40, 41, 43, and 44.

Among the claims that are currently in the case, the following claims are presented herein as originally filed: Claims 2 to 6, 8, 16, 19 to 22, 24, 25, 28, 29, 35, 38, and 39.

Among the claims that are currently in the case, the following claim is currently amended herein: Claim 1.

Walters et al
Ser. No. 10/537,254
Docket No. 05-084
AMENDMENT

No additional fee is required with respect to the claims in the case.

A PETITION FOR REQUEST FOR EXTENSION OF TIME to extend the time to respond by ONE Month to 05/06/2009, along with an extension fee, is filed concurrently herewith.

In view of the foregoing, it is respectfully requested that claims 1 to 6, 8, 16, 19 to 22, 24, 25, 28, 29, 31, 35, 38, 39, 42, and 45 be deemed allowable. If the Examiner believes otherwise, or has any comments or questions, or has any suggestions for putting the case in condition for final allowance, the Examiner is respectfully urged to contact the undersigned attorney of record at the telephone number below, so that an expeditious resolution may be effected and the case passed to issue promptly.

It is noted that Exhibit A is attached hereto.

Respectfully submitted,

May 6, 2009
Date

Marvin S. Townsend
Marvin S. Townsend
Registration Number 27,959
Attorney for Applicant

Marvin S. Townsend
Patent Attorney
8 Grovepoint Court
Rockville, MD 20854
(Voice and Fax) 301-279-0660
E-mail: MTowsend@aol.com

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Walters et al
Ser. No. 10/537,254
Docket No. 05-084
AMENDMENT

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